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Title: Decadal-Scale Litter Manipulation Alters the Biochemical and Physical Character of Tropical Forest Soil Carbon

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Abstract

Climate change and rising atmospheric carbon dioxide (CO_2) concentrations are likely to alter tropical forest net primary productivity (NPP), potentially affecting soil C storage. We examined biochemical and physical changes in soil C fractions in a humid tropical forest where litter manipulation changed total soil C stocks. We hypothesized that: (1.) low-density soil organic C (SOC) fractions are more responsive to altered litter inputs than mineral-associated SOC, because they cycle relatively rapidly. (2.) Any accumulation of mineral-associated SOC with litter addition is relatively stable (i.e. low leaching potential). (3.) Certain biomolecules, such as waxes (alkyl) and proteins (*N*-alkyl), form more stable mineral-associations than other biomolecules in strongly weathered soils. A decade of litter addition and removal affected bulk soil C content in the upper 5 cm by +32% and –31% in total quantity, respectively. Most notably, C concentration in the mineral-associated SOC fraction was greater in litter addition plots relative to controls by 18% and 28% in the dry and wet seasons, respectively, accounting for the majority of increased bulk soil C stock. Radiocarbon and leaching analyses demonstrated that the elevated mineral-associated SOC consisted of new and relatively stable C, with only 3% of mineral-associated SOC leachable in salt solution. ^{13}C NMR spectroscopy indicated decadal-scale stability of waxes (alkyl C) and microbial biomass compounds (*O*-alkyl and *N*-alkyl C) in mineral-associated SOC with litter removal, and losses plant-derived compounds (aromatic and phenolic C). We conclude that shifts in tropical forest NPP, as will likely occur with global change, are likely to alter the quantity, biochemistry, and stability of C stored in strongly weathered tropical soils.

1. Introduction

Soil organic carbon (SOC) storage in humid tropical forests is likely to be affected by shifting net primary productivity (NPP) in response to global change. Remote sensing data show increased NPP for much of the tropics during recent decades in response to warming, increased rainfall (Nemani et al., 2003), and elevated atmospheric carbon dioxide (CO_2) (Lewis et al., 2009). At the same time, field studies have observed that warming (Clark et al., 2013) and drying (Tan et al., 2013) can suppresses NPP in tropical forests. This spatial variation may result from increased dry season duration in some areas (Boisier et al., 2015), versus increased wet season rainfall in other areas with climate change (Feng et al., 2013). Rainfall seasonality, in turn, is related to intra-annual shifts in SOC stocks, as shown for a humid forest in Panama (Turner et al., 2015). Since ~30% of global SOC stocks are in tropical forests (Jobbagy and Jackson, 2000), potential interacting effects of altered NPP and seasonality are of broad significance.

Changes in NPP are most likely to affect SOC storage via altered litterfall and/or root turnover. A global meta-analysis of 70 litter addition experiments showed a 31% overall increase in SOC content to 5 cm depths, with the largest changes in six tropical or sub-tropical studies included in the study (Xu et al., 2013b). More recently, litter manipulation in a humid Panamanian forest also resulted in significant changes in SOC stocks to 20 cm depths (Tanner et al., 2016). However, we know very little about how altered litterfall might affect the biochemistry, physical character, and stability of tropical SOC.

A useful conceptual model divides SOC into three fractions (Sollins et al., 1999): (1) free-debris SOC, which visually resembles decomposing litter and roots, is unattached to mineral particles, and tends to have younger C than other fractions in tropical soils (Marin-Spiotta et al., 2008); (2) occluded-debris SOC, which is litter that has been incorporated into aggregate

structures, or which is otherwise easily dispersible from soil; (3) mineral-associated SOC, often referred to as the "heavy fraction," which consists of organic compounds sorbed to mineral surfaces via physiochemical mechanisms (Kleber et al., 2015). Studies of clay-rich tropical soils have found that the majority of SOC resides in the mineral-associated fraction (Trumbore, 1993; Glaser et al., 2000; Marin-Spiotta et al., 2009), which is generally thought to be the most stable, long-term SOC stock (Trumbore and Zheng, 1996; Torn et al., 1997; von Lutzow et al., 2006). Recent evidence, however, suggests that the mineral-associated SOC fraction can contain heterogeneous sub-fractions that turn over on different timescales (Sollins et al., 2006; Cusack et al., 2011b), with certain compounds like proteins comprising the most stable portion of mineral-associated SOC (Kleber et al., 2007). In humid tropical forests in particular waxy substances and other lipids may also contribute to more stable soil fractions (Cusack et al., 2011a).

Here, we investigate the biochemistry, physical character, and stability of SOC after a decade of litter manipulation in a humid Panamanian forest, following up on documented shifts in SOC stocks with litter addition or removal at these sites (Tanner et al., 2016). We measured three SOC fractions in surface soils, where the greatest changes in SOC stocks were documented, during a wet and a dry season. We hypothesized that: (1) changes in bulk SOC concentrations with litter manipulation are explained by shifts in rapidly cycling free-debris and occluded-debris SOC fractions, because these are likely to change over short timescales; (2) the quantity and chemical character of mineral-associated SOC is relatively insensitive to decadal-scale changes in litter inputs; (3) any new SOC accumulation in mineral-associated soil fraction with litter addition is relatively stable (i.e., low solubility); (4) waxy (alkyl) and protein-rich (*N*-alkyl) C groups represent the most stable portion of mineral-associated SOC, persisting on decadal

timescales even in the absence of new litter inputs. Finally, (5) seasonal shifts in SOC result primarily from changes in the free-debris and occluded-debris SOC fractions.

2. Materials and Methods

2.1 Site and Soil Collection

Research was conducted in a lowland semi-deciduous tropical forests on the Gigante Peninsula in the Barro Colorado Nature Monument, Panama (9°06'N, 79°54'W) (Windsor, 1990). The forest is a tropical moist forest *sensu* Holdridge et al. (1971). The site has a tropical monsoon seasonal climate, mean annual rainfall is 2600 mm, and mean annual temperature is 26°C (Leigh, 1999). High tree species richness and the stature (canopy heights > 35 m) of this forest are characteristic of an old (> 200 years) seasonal primary forest (Leigh et al., 1996). Litterfall peaks during the dry season from December to April, and decreases by 50% during the wet season (Turner et al., 2015) Soils are moderately acidic (pH 4.8 – 5.4) kaolinitic Oxisols developed on basalt (Yavitt et al., 2009). The soils lack a distinct O horizon other than a continuous cover of leaf litter that builds up during the dry season and is virtually fully decomposed by the end of the wet season. Soils were classified in profile pits outside of plots according to Soil Taxonomy (SoilSurveyStaff, 1999). Soil were well-structured, with medium and coarse subangular blocky structure in the upper parts of the profile, breaking to strong fine subangular blocky. Some shrink-swell clays are evident in surface soils at these sites, possibly resulting from African dust inputs (Gross et al., 2016).

Fifteen 45 × 45 m litter manipulation plots were established in 2000 and trenched to a depth of 50 cm. The sides of the trenches were lined with heavy plastic. This depth was chosen to contain fine roots and deter growth into or out of plots, since >92% of fine root biomass is in

the top 50 cm of soil in forests in this region (Yavitt and Wright, 2001; Cusack et al., 2018). Monthly transfer of litter from five removal plots to five addition plots began in January 2003, and five control plots were included (n = 5). Litter was removed by rake and by hand monthly and evenly distributed onto the litter addition plots. A study to 1-m depth after 6 years of litter manipulation at these sites showed that litter addition and removal significantly increased and decreased bulk soil C stocks to 20 cm depth, respectively, with the greatest effect in the upper 5 cm of the profile (Tanner et al., 2016). This was consistent with an earlier study at 3 years showing significant treatment effects on organic matter concentrations in the top 2 cm of soils at the site (Vincent et al., 2010). Our goal was to better understand the physiochemical nature of the changing SOC stocks documented in these previous studies.

Soils for this study were collected during the dry season in February 2013, and the wet season in July 2013. Soils were sampled from the surface 5 cm using a 2.5 cm diameter constant-volume soil corer, thus sampling equivalent depths and volumes in all treatments. Surface litter was gently removed and coring began at the surface of the mineral soil. In litter addition plots a spatially heterogeneous duff layer had developed, and this was included in sampling where it was present. Three soil cores were sampled from 10 random stratified points within the 30 x 30 m core area of each plot, then pooled and homogenized to give one representative sample per plot. The 0 – 5 cm depth was selected because it showed the strongest changes in bulk soil C with litter manipulation at 6 years (Tanner et al., 2016), and this depth has shown the strongest changes in litter manipulation experiments more broadly (Xu et al., 2013a).

Bulk density cores were collected using a 5 cm diameter corer in each plot. Gravimetric soil moisture, pH, bulk density, and SOC fractions (see below) were measured on fresh soils. Loss on ignition (LOI) was also measured for dry season samples to assess changes in the

mineral content of soils across treatments to the same depth. Carbon content normalized to mineral mass was assessed as an alternative to the strategy used here of collecting samples from a constant depth and volume across plots.

Given the previously documented change in bulk density with litter removal at these sites (Tanner et al., 2016), an alternative sampling approach could have been to adjust sampling depth in an attempt to collect equivalent mass. Soil volume increases and bulk density declines as organic matter is added. Thus, we would have had to sample to shallower depths in litter removal plots and deeper depths in litter addition plots to collect equivalent mass. This would pose problems because the composition of SOC changes with depth. For example, the proportion of dense, mineral-associated SOC increases with depth. We therefore determined that sampling equivalent mass would have masked any increased importance of low-density organic matter in surface soils, as reviewed by Sollins and Gregg (2017). Since our goal was to characterize the physiochemical characteristics of the change in surface SOC including chemical shifts related to changes in bulk density, we decided to sample to constant depth and volume rather than correct for changes related to bulk density.

2.2 Soil Fractionation

Density fractionation was used to separate soils into three soil SOC pools using a solution of C-free sodium polytungstate (SPT) following Swanston et al. (2005), with modifications as noted. This method was chosen over particle size fractionation because it puts greater emphasis on adsorption and aggregation processes, rather than the fate of particulate organic matter (Moni et al., 2012). The density fractions included: (**1**) free-debris SOC, which was collected as the fraction of the soil that easily floated in dense liquid with minimal disturbance (sometimes

referred to as the "free light fraction" in the literature). (**2**) Occluded-debris SOC, which was collected as floatable SOC that was released from soil via mixing and dispersed via sonication at 450 J/mL (sometimes referred to as the "occluded light fraction"). This SOC fraction is generally considered to turnover more slowly that the free-debris SOC fraction because of its physical protection with aggregate structures, as shown in an Australian Oxisol (Golchin et al., 1995). This fraction may also represent SOC at more advanced stages of decomposition than the free-debris, as suggested by elevated alkyl:*O*-alkyl ratios (Golchin et al., 1994). However, the quantity and chemistry of SOC released via sonication can be highly variable across soils (Wagai et al., 2009), and may include debris buried inside macro-aggregates, debris entrained in the organic coatings that have built up at the surface of micro-aggregates, and/or individual organic particles released from mineral grains by sonication. Iron-oxide particles in Oxisols are often bound up in exceptionally stable micro-aggregates (<5 um), and these were not likely broken down by the method used here. (**3**) Mineral-associated SOC, which was collected as the dense pellet of soil remaining after removing the above two fractions (sometimes referred to as the "dense" or "heavy" fraction). This fraction generally represents the most stable and oldest soil C. Separation of fractions was done in sodium polytungstate (SPT) mixed to a density of 1.7 g/cm^3, following initial tests to identify optimal density and dispersal parameters (Appendix S1).

Bulk soils and SOC fractions were analyzed for C and N concentrations on a Costech Elemental Analyzer (Valencia, CA) using acetanilide as a standard. While the focus of this paper is on SOC, we included parallel N analyses to understand broader effects of litter manipulation on soil organic matter (SOM) N content and C:N ratios, particularly in relation to SOM protein content versus other C functional groups.

2.3 $\Delta^{14}C$ and ^{13}C NMR of Mineral-Associated SOC

Because the mineral-associated SOC fraction commonly contains the longest-term soil C sink (Sollins et al., 1999), we conducted extensive chemical analysis of this C fraction to determine the potential effects of changing NPP on long-term tropical soil C storage.

First, we measured the ^{14}C content of the mineral-associated SOC fraction to assess relative changes in the overall age of this C fraction with litter addition and removal. The spike in atmospheric $^{14}CO_2$ from nuclear bomb testing in the 1960s and subsequent declines make it possible to assess relative ages of plant organic matter inputs to ecosystems on decadal timescales (Trumbore, 2000). The mineral-associated SOC fraction was measured for $\Delta^{14}C$ at the WM Keck Carbon Cycle Accelerator Mass Spectrometry (AMS) Laboratory at UC Irvine following sample graphitization (Vogel et al., 1984). AMS analytical precision was ± 2.2 ‰ for these samples, and results are reported as the per mil (‰) deviation from a standard normalized for ^{13}C.

We also assessed changes in the organic chemistry of the mineral-associated SOC fraction to understand how litter addition and removal altered this important C fraction. The organic chemistry of the mineral-associated fraction was assessed using ^{13}C nuclear magnetic resonance (NMR) spectroscopy following hydrofluoric acid treatment (details in Appendix S1), which removes mineral and glass fractions to reduce magnet interference, and concentrates SOC to reduce noise in the spectra (Schmidt et al., 1997). This method also removes a portion of acid-soluble C as DOC from mineral associations (Gelinas et al., 2001), leaving a sample of mineral-free, concentrated organic matter that is not soluble in hydrofluoric acid. Here, the hydrofluoric-acid-insoluble portion of mineral-associated SOC was 60.5% of the C contained in the mineral-associated fraction (Table S6), which is within the range found for a comparison of

196 different soil types (Hockaday et al., 2009). The mineral-associated SOC remaining after
197 hydrofluoric acid treatment is the least dynamic, least soluble component of this pool.
198 Cross polarization ^{13}C NMR was conducted on the concentrated SOC after hydrofluoric
199 acid treatment using a 300 MHz Bruker Avance III NMR spectrometer (Bruker BioSpin,
200 Billerica, MA) at Baylor University (Waco, TX). The 4mm sample probe was operated at a
201 magic angle spinning (MAS) frequency of 12 kHz (details in Appendix S1). Resulting spectra
202 were assessed for the relative contributions of seven C functional groups by integrating the signal
203 magnitude on the δ–scale. Carbon functional groups are followed in parentheses by some of the
204 many common C compounds in which they occur: (1.) alkyl 0 to 45 ppm (e.g., waxes, other
205 lipids); (2.) *N*-alkyl+methoxyl (hereafter *N*-alkyl) 45 to 60 ppm (e.g., proteins, peptides,); (3.) O-
206 alkyl 60 to 95 ppm (e.g., cellulose, other carbohydrates); (4.) di-O-alkyl 95 to 110 ppm (e.g.
207 hemicellulose); (5.) aromatic 110 to 145 ppm (e.g., lignin, tannin); (6.) phenolic 145 to 165 ppm
208 (e.g., acids, tannin (Min et al., 2015)); and (7.) amide+carboxyl 165 to 215 ppm (e.g., chitin,
209 proteins, peptides, and hemicellulose) (Li et al., 2015). The alkyl:*O*-alkyl ratio was calculated as
210 an index of decomposition stage, with greater ratios generally indicative of later stages of litter
211 decomposition (Preston et al., 2009).
212
213 ***2.4 Soil C Stability and DOC Leaching***
214 The stability of C was assessed in each SOC density fraction by measuring the solubility
215 of organic matter as DOC and TDN leached in the SPT supernatant plus approximately 1-L of
216 deionized water (DI). This approach specifically mobilized DOC from cation and anion
217 exchange sites, in an analogous approach to the commonly used potassium chloride (KCl) salt
218 extractions for collecting DOC from soil (Swift, 1996). Floatable debris fractions were rinsed

with DI in filter holders through 0.4 μm membrane filters until approximately 1-L was reached, which both cleaned the fraction of SPT, and allowed us to conduct the leaching experiment. The dense fraction was rinsed with DI in centrifuge tubes by shaking, centrifuging, decanting of supernatant. Decanted supernatant was then filtered as above, and any residue on the filter was added back to the dense fraction. Initial rinses included removal of the SPT from the fraction. Thus, all C in initial bulk soils was accounted for either in SOC solid fractions, or in leachate from the fractionation procedure and subsequent leaching (details in Appendix S1). Leachate samples were analyzed on a Shimadzu TOC-L with TDN analyzer (Columbia, MD).

2.5 Statistical Analyses

Data from the litter manipulation plots were analyzed using analysis of variance (ANOVA) and analysis of covariance (ANCOVA). The effects of season, treatment, and their interaction were tested for bulk soil C and N content, C and N in each density fraction, ^{14}C content of the mineral-associated fraction, and for ^{13}C NMR chemical regions of the mineral-associated fraction. Effects of season and treatment were also tested for DOC and TDN leached from each SOC fraction. We used post-hoc Tukey HSD means separation tests to assess differences among litter treatments and seasons (n = 5 per season and litter treatment). Post-hoc regressions were used to determine relationships between continuous variables across seasons (n = 30 for bulk soils, 30 for each density fraction). We used JMP 11 statistical software (SAS Institute). Data are presented as means ± 1 S.E., using $p < 0.05$ for statistical significance.

3. Results

After 10 years of litter manipulation, bulk soil C concentrations from 0 – 5 cm depths

were significantly greater with litter addition (+32%), and significantly lesser with litter removal (-31%) relative to control plots (Figure 1a, Table 1). Similar significant effects were observed for soil N concentrations, but the magnitude of the differences were not as large, so soil C:N values were significantly affected by treatment, with the lowest levels in litter removal plots (Table 1).

Bulk density was also significantly greater in litter removal plot (0.97 ± 0.05 g/cm^3) relative to the other treatments, while there was no significant difference between control (0.73 ± 0.03 g/cm^3) and litter addition plots (0.79 ± 0.05 g/cm^3, n = 5), and no seasonal effect (Table 1). This change represents a relatively large (+28%) increase in bulk density with litter removal relative to the other two treatments. Our approach of sampling equivalent depths and volumes across treatments explicitly did not correct for changes in bulk density, since these changes were due to addition of low-density organic matter, which we did not want to dilute by sampling to greater depth. Nonetheless, we can evaluate the effect of the change in bulk density on our bulk soil C content values. Using published data from our sites for C concentration versus depth to 200 cm at six years (Tanner et al., 2016), we can calculate the depths we would have had to sample, and the resulting effect on bulk soil C content. The 28% greater bulk density that we observed with litter removal relative to controls is equivalent to a 1.4 cm increase in effective soil depth. Using the published soil depth profiles, we calculate that this greater effective soil depth would lead to a 2.4 % increase in C for litter removal plots relative to control plots, if our goal had been to calculate C stocks on a per-mass of soil basis. This is a small proportion of our observed 31% decline in bulk soil C concentration with litter removal.

Another approach is to normalize soil C content to mineral content. Loss on ignition values were significantly lower in litter removal plots (17.5 ± 0.3 %) relative to other treatments, indicating greater content of minerals/ash, and there was no significant difference between

control and litter addition plots (19.7 ± 0.3 % and 20.6 ± 0.8, respectively, n = 5). Using these LOI values and bulk density to standardize soil C content to mineral content for 0 – 5 cm depth, there was significantly greater soil C content per mineral content in litter addition plots (98.5 ± 4.5 mg-C/g-mineral-content) versus control plots (75.3 ± 3.0 mg-C/g-mineral-content), with the lowest values in litter removal plots (49.5 ± 2.5 mg-C/g-mineral-content, $p < 0.05$). Thus, normalizing soil C to mineral content gave similar differences between addition vs. control (+31%), and removal vs. control (-34%) as did C concentration and C stock data sampling equivalent volume (Table 1).

3.1 Soil Fraction C Contents, Relative Masses, and C Concentrations

Soil organic C content was greater with litter addition versus controls and litter removal for all three soil fractions (Figure 1), with accompanying changes in C concentrations within each fraction (Table S2), and changes in the relative masses of the three fractions (Figure S2a), such that the contribution of each fraction to total soil C shifted significantly with litter manipulation (Figure S2b). There were also seasonal effects on all fractions, with interactions between season and treatment effects. The most important effect of litter manipulation on the mineral-associated SOC fraction, in terms of the net effect on bulk soil C content, was a change in C concentration within this fraction. In contrast, the most important effect on the free-debris and occluded-debris SOC fractions was an increase in the relative mass of these fractions as a proportion of total soil mass with litter addition. Results for the mineral-associated fraction are presented first, followed by the two floatable fractions together.

3.1.1. Mineral-Associated SOC Fraction

The mineral-associated SOC fraction comprised the largest portion of greater bulk soil C with litter addition (Figure 1a). The changes in mineral-associated soil C content resulted from greater C concentrations in this soil fraction with litter addition, and lesser C concentrations with litter removal (Table S2). Even though average C concentrations for the mineral-association SOC fraction were low relative to the other two fraction (3 – 5% C in mineral-association SOC, versus 20 – 40% C in the floatable fractions, Table S2), the large relative mass of this soil fraction (91 – 97 % of soil mass, Figure S2a) meant that it dominated net changes in soil C content. So, even with the relatively low average C concentrations, the mineral-association SOC fraction contributed the largest overall proportion of soil C content, containing 64 – 82 % of total soil C (Figure S2b).

There was also a seasonal effect on C concentrations within the mineral-associated fraction, with greater C concentrations overall and larger differences among treatments during the wet season (Figure 1a). Specifically, mineral-associated C concentrations were 4.0 ± 0.2 % in control plots versus 4.7 ± 0.2 % in litter addition plots during the dry season (Table S2), representing an 18% increase. During the wet season, mineral-associated C concentrations were 4.3 ± 0.1 % in control plots, versus 5.5 ± 0.2 % in litter addition plots (Table S2), representing an 28% increase in C concentration. Mineral-associated C concentrations in litter removal plots were 25% lower than control plots the dry season, and 28% lower than control plots during the wet season (Table S2). Nitrogen concentrations in the mineral-associated soil fraction showed similar significant shifts with litter removal, but the magnitude of changes in N concentration were not as large, so C:N ratios in the mineral-associated SOC fraction were lower in litter removal plots than in litter addition plots, indicating a relative enrichment in N with litter removal (Table S2).

311 Assessing the total C content within the mineral-associated fraction, using C
312 concentrations and the mass of this fraction as a proportion of soil mass, there was 43.5 ± 2.1
313 mg-C/g-bulk-soil in mineral-associated SOC in litter addition plots, 38.7 ± 1.7 mg-C/g-bulk-soil
314 in control plots, and 28.9 ± 1.6 mg-C/g-bulk-soil in litter removal plots in the dry season (Figure
315 1a). In the wet season there was 50.7 ± 2.0 mg-C/g-bulk-soil in the mineral-associated SOC
316 fraction in litter addition plots, 41.0 ± 1.1 mg-C/g-bulk-soil in control plots, and 30.2 ± 1.5 mg-
317 C/g-bulk-soil in litter removal plots (Figure 1a). Thus, litter addition plots had 12% greater
318 mineral-associated SOC content than controls during the dry season, and 24% greater during the
319 wet season relative to controls. Litter removal plots showed less of a seasonal effect on mineral-
320 associated SOC content, with removal plots 25% lesser than controls during the dry season, and
321 26% lesser during the wet season relative to controls. Comparing shifts from the dry season to
322 the wet season, mineral-associated SOC content increased 17% in the litter addition plots from
323 the dry to the wet season, 6% in control plots, and only 4% in litter removal plots. These
324 seasonal shifts illustrate the interaction between litter treatment and season, with wet season
325 increases in mineral-associated SOC content much greater in litter addition versus litter removal
326 plots.
327
328 ***3.1.2. Low-Density SOC Fractions***
329 Unlike the mineral-associated SOC fraction, the change in soil C content attributable to
330 the two floatable fractions (Figures 1b and 1c) was related primarily to shifts in their relative
331 masses within soil (Figure S2a), rather than large changes in their C concentrations (Table S2).
332 Nonetheless, there were significant but small effects of litter manipulation and season on C

concentration in the free-debris SOC fraction (Table S2). The occluded-debris fraction had no significant effect of litter manipulation on C concentrations.

The shift in the relative masses of these fractions with litter manipulation was quite large. For the free-debris SOC fraction, relative mass was 89% and 37% greater in litter addition plots relative to controls during the dry and wet seasons, respectively (Table S2, Figure S2a), and -63% and -58% lesser in litter removal plots relative to controls during the dry and wet seasons, respectively. The occluded-debris SOC fraction did not show strong treatment effects during the dry season, but during the wet season its mass was 74% greater in litter addition plots relative to controls, and -18% lower in litter removal plots relative to controls (Table S2, Figure S2a). Given the sizeable shifts in the proportional masses of the two low-density fractions with litter manipulation, and their large C concentrations, their overall contributions to total soil C content was significant, even though their proportional masses were small, contributing only 1-6% of total soil mass (Table S2).

Using C concentration values and soil mass proportions to calculate the C content in the two low-density SOC fractions, the free-debris SOC fraction had 17.7 ± 3.4 mg-C/g-bulk-soil in litter addition plots, 9.2 ± 1.0 mg-C/g-bulk-soil in control plots, and 3.4 ± 0.5 mg-C/g-bulk-soil in litter removal plots in the dry season (Figure 1b). In the wet season, there was 10.3 ± 2.4 mg-C/g-bulk-soil in litter addition plots, 7.2 ± 1.0 mg-C/g-bulk-soil in control plots, and 2.6 ± 0.3 mg-C/g-bulk-soil in litter removal plots for the free-debris SOC fraction (Figure 1b). This represents 92% greater free-debris C content in litter addition plots versus controls in the dry season, and 43% greater free-debris C content in litter addition plots versus controls during the wet season, as well as 63% less in litter removal plots versus controls during the dry season, and 64% less in litter removal plots versus controls during the wet season.

In the occluded-debris SOC fraction, there was 6.1 ± 1.1 mg-C/g-bulk-soil in litter addition plots, 3.6 ± 1.1 mg-C/g-bulk-soil in control plots, and 3.8 ± 0.5 mg-C/g-bulk-soil in litter removal plots in the dry season (Figure 1a). In the wet season, there was 12.1 ± 3.5 mg-C/g-bulk-soil in litter addition plots, 5.8 ± 1.8 mg-C/g-bulk-soil in control plots, and 3.9 ± 1.0 mg-C/g-bulk-soil in litter removal plots for the free-debris SOC fraction (Figure 1c). This represents 69% more occluded-debris C in litter addition plots versus controls during the dry season, and 108% more occluded-debris C in litter addition plots versus controls during the wet season. There was no significant decrease in occluded-debris C content with litter removal in the dry season, but there was 33% less occluded-debris C content in litter removal plots versus controls during the wet season.

There were also overall seasonal effects on the proportion of soil mass and the proportion of C content for the two low-density SOC fractions. The free-debris SOC fraction was a larger proportion of total soil C (Figure S2b) and of total soil mass (Figure S2a, Table S2) during the dry versus wet season. In contrast, the occluded-debris fraction represented a significantly larger proportion of total soil C (Figure S2b) and of total soil mass (Table S2) during the wet versus dry season. Thus, seasonal patterns for C content in the occluded-debris fraction mirrored patterns for the mineral-associated SOC fraction, and the occluded-debris fraction had opposite seasonality (Figure 1).

3.2. Organic Chemistry and Δ ^{14}C Age of Mineral-Associated SOC

The Δ ^{14}C of the mineral-associated SOC fraction was significantly greater with litter removal versus addition, with no seasonal effect (Figure 2). Because the Δ ^{14}C signal in surface soil is dominated by recent C inputs from plant litter, the greater values in litter removal plots

correspond to an older overall age of the mineral-associated SOC fraction, versus a younger overall mineral-associated SOC fraction in litter addition plots with greater dominance by recent litter inputs.

Solid-state ^{13}C NMR spectroscopy revealed decadal-scale effects of litter manipulation on the organic chemistry of mineral-associated SOC, with no seasonal effects. All C groups responded to litter manipulation except amide+carboxyl C. Overall, alkyl C, *N*-alkyl C, and *O*-alkyl C were proportionally larger components of mineral-associated SOC in litter removal versus addition plots, while di-*O*-alkyl C, aromatic C, and phenolic C showed the opposite pattern (Table 2). These changes resulted in an overall shift in the chemical nature of mineral-associated SOC, as illustrated by comparing representative spectra from litter addition and removal plots (Figure 3). Thus, there was a significant treatment effect on the ratio of (alkyl + *O*-alkyl + *N*- alkyl):(phenolic + aromatic) C (Table 2). There was also a significant interaction between season and treatment only for the alkyl:*O*-alkyl ratio in mineral-associated SOC, with significantly greater ratios in litter addition plots (1.5 ± 0.04) versus control (1.4 ± 0.03) and removal plots (1.3 ± 0.02) during the dry season only, and no effect during the wet season. This dry season pattern is the opposite pattern of what would be expected for more advanced stages of decomposition in litter removal plots. Overall, these alkyl:*O*-alkyl ratio for mineral-associated SOC were all significantly greater than values obtained for occluded-debris SOC at varying sonication energy levels, suggesting that our method appropriately separated these two fractions (Appendix S1).

Two C chemical groups were significantly related to the Δ ^{14}C content of mineral-associated SOC. Mineral-associated Δ ^{14}C was positively significantly correlated to *O*-alkyl C (R^2 = 0.18, n = 30), indicating older C present when more *O*-alkyl C was present. In contrast,

mineral-associated $\Delta\ ^{14}C$ was negatively significantly correlated to phenolic C (R^2 = 0.27, n = 30), indicating younger C present when more phenolic C was present.

3.3. SOC Stability: DOC Leaching

The proportion of salt-extractable C (i.e., leachable DOC) in each soil fraction, expressed as mg-C-leached/g-C retained, varied across SOC fractions, with no effect of season. Overall, DOC leaching was 5–14 % of the stable C in the free-debris ("free floatable") fraction, 15–30 % of the stable C in the occluded-debris ("dispersible floatable") fraction, and only 2–3 % of the stable C in the mineral-associated ("dense") fraction (Figure 4, Table S3). Thus, all SOC fractions had a component that was salt-extractable, but this proportion was smallest in the mineral-associated fraction, indicating that more of the C in this fraction was relatively stable.

The effect of litter manipulation on the proportion of C that was salt-extractable varied across SOC fractions. In the free-debris fraction, a significantly greater proportion of DOC was leachable from litter removal plots (12.7 ± 0.8 %) relative to control (6.2 ± 0.6 %) or litter addition plots (4.9 ± 0.4%), suggesting a high level of instability and solubility for the small stock of free-debris organic matter remaining in litter removal plots after a decade (Figure 4a). In contrast, the mineral-associated SOC fraction had a significantly greater proportion of leachable DOC in litter addition plots (2.9 ± 0.3 %), relative to control (2.3 ± 0.2 %) or litter removal plots (1.8 ± 0.1 %, Figure 4b), indicating that C remaining in mineral associations in litter removal plots after a decade was strongly sorbed to mineral surfaces and stable. Significant treatment effects on proportional losses of total dissolved N (TDN) were similar to those for DOC, although N was much more mobile overall than C (Table S3).

Overall, the proportion of C that was leachable as DOC from the mineral-associated

fraction was positively correlated with the total quantity of C in that fraction (R^2 = 0.66, $p < 0.05$, n = 30). Accordingly, bulk quantities of leachable DOC per g soil showed similar treatment effects as did soil C concentrations across fractions (Figures S4 versus Figure 1, Table S2), and so did trends for leachable TDN per g soil (Tables 2, S5). Total DOC leaching was 1.1 ± 0.2 mg-C/g-bulk-soil in litter addition plots, 0.9 ± 0.1 mg-C/g-bulk-soil in control plots, and 0.5 ± 0.04 mg-C/g-bulk-soil in litter removal plots in the dry season (Figure 1a). In the wet season DOC leaching was 1.6 ± 0.3 mg-C/g-bulk-soil in litter addition plots, 0.9 ± 0.1 mg-C/g-bulk-soil in control plots, and 0.6 ± 0.1 mg-C/g-bulk-soil in litter removal plots (Figure S4). These quantities represent very small fractions of total soil C content (Figure 1), ranging from just 1.7 – 3.1 % of total soil C leachable as DOC.

4. Discussion

The effects of litter manipulation on bulk soil C concentrations after 10 years in these Panamanian forests indicate a sustained effect, with similar effects observed from 0 – 2 cm depth after three years (Vincent et al., 2010), and from 0 – 20 cm depth after six years (Tanner et al., 2016). The increase in soil C content with litter addition occurred despite an apparent priming effect of added litter on soil CO_2 fluxes at these sites (Sayer et al., 2011). Similar to our ±30% change in bulk soil C content with litter addition or removal, two years of litter manipulation in a Costa Rican forest on Ultisols also changed bulk soil C concentrations by approximately ±30% (Leff et al., 2012). In contrast, a review of litter manipulations in temperate forests showed generally non-significant or small effects on bulk soil C content (Sayer, 2006). Our results, in conjunction with Leff et al. (2012). and the few tropical studies in the meta-analysis (Xu et al., 2013b), suggest that soil C stocks in strongly weathered tropical forest soils may be more

sensitive to changes in NPP over shorter time periods than soils in temperate sites. Such a trend may reflect faster C cycling, greater overall C inputs to soils per year, and/or sorptive characteristics of the strongly weathered clay soils common in humid tropical forests.

4.1. Elevated SOC across Soil Fractions with Litter Addition

The substantial accumulation of organic matter that we observed in the mineral-associated SOC fraction was somewhat surprising, since this soil fraction is commonly a relatively stable pool of long-term soil C storage (Schmidt et al., 2011). Elevated mineral-associated SOC may have resulted in part from the elevated soil pH observed in litter addition plots (Tanner et al., 2016), since increased pH can mobilize DOC from organic matter (Whitehead et al., 1981), transporting C from litter down into mineral soils. Mobilized DOC may then have sorbed to the relatively large number of binding sites common on clay surfaces in highly weathered tropical forest soils (Chorover and Sposito, 1995). Or, DOC may have associated with other organic compounds already sorbed to mineral surfaces (Kleber et al., 2007), or to surfaces of very stable microaggregates (< 1 μm diameter) (Torrent et al., 1990).

The low-density SOC fractions also accumulated in soils with litter addition, as expected, indicating that decomposition rates and other C losses did not accelerate to balance new litter inputs. The greater levels of occluded-debris SOC in litter addition plots could have resulted from organic matter incorporation into aggregate structures, particularly during formation and dissolution of aggregates over the course of rainfall seasonality (Dimoyiannis, 2009). In contrast to our results, a 50-year experiment in two temperate forests on silt-loam soils with lower clay content found that litter addition increased only the low-density SOC fractions, while litter removal reduced both low-density and mineral-associated SOC fractions (Lajtha et al., 2014). A

471 better understanding of the ecosystem and soil characteristics that promote rapid accumulation of
472 organic matter into stable mineral associations could help us better predict C cycle responses to
473 global change across biomes.
474
475 ***4.2. Stability of C in SOC Fractions: Potential DOC Leaching***
476 As hypothesized, C incorporated into the mineral-associated soil fraction was
477 subsequently relatively stable, as demonstrated by its low solubility and leaching potential.
478 Similarly, a range of soil orders from North and Central American sites showed rapid
479 incorporation of added ^{15}N into the mineral-associated fraction in a laboratory study, with
480 subsequent low levels of solubility (Strickland et al., 1992). A desorption study using goethite
481 and a subsoil similarly showed <3% desorption of organic matter when leaching solution was
482 similar to sorption solution, and elevated desorption only under elevated ion concentrations or
483 elevated pH (Kaiser and Zech, 1999). Thus, it appears that in one direction organo-mineral
484 associations can be highly dynamic (e.g., rapid mineral sorption of organic compounds), but in
485 the other direction, losses from this pool are more limited (i.e., not leachable as DOC),
486 illustrating how this fraction can be a reservoir of long-term soil C storage. The longer-term
487 stability of elevated mineral-associated SOC in litter addition plots merits further investigation.
488 Interestingly, the litter manipulation effect on DOC leaching as a proportion of extant
489 SOC was different for the two low-density SOC fractions compared with the mineral-associated
490 fraction. Litter removal versus addition plots had greater DOC leaching from low-density SOC
491 fractions, but lesser DOC leaching from mineral-associated SOC fractions. This opposite
492 treatment effect likely reflects the different physiochemical nature of organic matter in these
493 different SOC fractions. Organic matter in the low-density fractions is comprised primarily plant

494 or microbial macro tissues at some stage of decomposition, whereas the mineral-associated SOC
495 fraction contains small organic compounds sorbed to mineral surfaces out of DOC. Thus, high
496 levels of DOC leaching from low-density SOC fractions in litter removal plots likely resulted
497 from the rapid dissolution of very decomposed macro tissues. In contrast, low levels of DOC
498 leaching from the mineral-associated DOC fraction in litter removal plots likely resulted because
499 the organo-mineral associations remaining after a decade of *in situ* leaching with rainwater were
500 the most stably sorbed portion of mineral-associations.
501
502 ***4.3. Changes in the $\Delta^{14}C$ of Mineral-Associated SOC***
503 Our radiocarbon data support growing evidence that only a portion of the mineral-
504 associated SOC fraction is stable over the long term, whereas another portion is dynamic on
505 decadal timescales. The relatively greater $\Delta^{14}C$ values of mineral-associated SOC in litter
506 removal plots suggest decadal-scale retention of relatively older C, assuming that the majority of
507 C in surface soils originated from NPP during the past 60 years (Trumbore, 2000). In contrast,
508 the lesser $\Delta^{14}C$ values in mineral-associated SOC in litter addition plots suggest an accumulation
509 of relatively recent C inputs. Because $\Delta^{14}C$ is an integrated measure of all C included in the SOC
510 fraction, the "modern" overall ages measured here do not preclude the presence of some much
511 older C. For example, mineral-associated SOC fractions of surface soils in Puerto Rico had $\Delta^{14}C$
512 values of 74–81 ‰, similar to values here, and turnover times calculated using archived samples
513 and a two-pool model showed that > 30% of the C had turnover times on the scale of millennia
514 (Hall et al., 2015). The mineral-associated SOC fraction in general may be more accurately
515 represented using a two-pool model (Torn et al., 2013). Thus, a portion of our mineral-associated
516 SOC fraction is likely very old, and the treatment effect we observed suggests that this older

517 portion was the most stable component of mineral-associated SOC.

518

519 ***4.4. Changes in the Organic Chemistry of Mineral-Associated SOC***

520 Mineral-associated SOC chemistry changed after a decade of litter removal, with
521 increased dominance of alkyl, *O*-alkyl, and *N*-alkyl C, which is likely indicative of the relative
522 stability of these C functional groups on mineral surfaces. The increased dominance of *N*-alkyl C
523 groups in litter removal plot mineral associations points to the importance of proteins. Proteins
524 have been put forth as a central component of stable mineral-associated SOC, because they sorb
525 strongly to mineral surfaces and have hydrophobic outward-facing protons, leading to further
526 associations with a wide range of organic compounds (Kleber et al., 2007). Several recent
527 laboratory and field studies also provide support for the central role of microbial proteins in the
528 formation of stable organo-mineral associations. One set of lab experiments showed rapid and
529 preferential sorption of microbial proteins to mineral surfaces (Swenson et al., 2015), and
530 another set showed that microbial biomass products dominated SOC accumulation in model soils
531 (Kallenbach et al., 2016). In the field, a European forest litter decomposition experiment
532 demonstrated that C from added litter became stably associated with mineral surfaces after
533 incorporation into microbial biomass (Hatton et al., 2012), and an isotope labeling experiment in
534 temperate grassland soils showed that soluble C compounds from litter were stored in SOC after
535 microbial uptake (Cotrufo et al., 2015). In our study, the lower C:N ratios we observed in
536 mineral-associated SOC with litter removal also suggest more advanced stage of microbial
537 processing (Baisden et al., 2002), and/or greater protein content. Together, these results supply
538 growing evidence that microbial biomass products like proteins play a dominant role in the
539 formation of stable, mineral-associated SOC.

Other C groups have also been posited as playing a role in long-term, stable C retention in soils. In particular, aromatic and phenolic compounds like lignin, as well as alkyl C, representative of waxes and other lipids, have been seen to be preferentially preserved in soils (Baldock et al., 1997; De Marco et al., 2012) (Baldock et al., 1992). However, there is conflicting data about the long-term stability of aromatic C in particular. For example, field data from cultivated soils demonstrated that added aromatic compounds (lignin) were lost relatively rapidly from soils, with no preferential retention (Gleixner and Poirier, 2001). More generally, there is growing consensus in the literature that few, if any, organic compounds are retained in soils over the long-term based solely on their chemical structure (i.e. without some form of physical protection like sorption to minerals) (Schmidt et al., 2011) (Lehmann and Kleber, 2015). For aromatic-rich compounds, desorption experiments suggest that the sometimes observed preferential retention of compounds like lignin may occur because aromatics can bind more strongly to mineral surfaces than other compounds in some cases (Kaiser and Zech, 1999; Kaiser, 2003). In our data, we saw no evidence for long-term retention of aromatic C in the mineral-associated SOC fraction. Also, the negative relationship between $\Delta\ ^{14}C$ and phenolic C in mineral-associated SOC indicated that phenolics contributed a relatively younger, more dynamic portion of this SOC fraction. We did see increased dominance of alkyl C with litter removal, as expected. The waxy, tough leaves characteristic of these Panamanian forests (Coley, 1983) likely had an influence on SOC chemistry (Kögel-Knabner, 2002). Alternately, alkyl C in soils may be more strongly related to soil microbial biomass rather than litter chemistry, as observed in subtropical plantations in China (Wang et al., 2015). Here, the increased dominance of alkyl C in litter removal plots was in mineral-associated SOC, suggesting that even this relatively recalcitrant C group depended on mineral associations for protection from

563 decomposition.

564 In contrast to alkyl C, *O*-alkyl C, representative of carbohydrates like cellulose, is
565 generally lost from litter quickly during microbial decomposition (Preston et al., 2009). Thus, the
566 ratio of alkyl:*O*-alkyl C has been used to measure the extent of microbial processing of organic
567 matter in litter and soil (Baldock et al., 1997; LaRowe et al., 2012; Keiluweit et al., 2016). It was
568 therefore surprising that the Panama litter removal plots had elevated proportions of both alkyl
569 and *O*-alkyl C in mineral-associated SOC, and significantly lower soil alkyl:*O*-alkyl ratios
570 relative to control plots. Also, the positive relationship between $\Delta^{14}C$ and *O*-alkyl C indicated
571 that *O*-alkyls contributed a relatively older, more stable portion of mineral-associated SOC.
572 Although unusual, preferential retention of *O*-alkyl C has been observed in some other forests,
573 particularly for plant litters with high alkyl:*O*-alkyl starting ratios (e.g. waxy leaves), and
574 resistant *O*-alkyl C compounds (e.g. from tannins) (Almendros et al., 2000). Also, cell wall
575 polymers in fungal and bacterial biomass (e.g., chitin and peptidoglycan) are comprised largely
576 of *O*-alkyl C plus proteins. Thus, the preferential retention of *O*-alkyl C that we observed with
577 litter removal may indicate a particularly resistant carbohydrate group from plant litter, but it
578 seems more likely, together with the *N*-alkyl data (above), that increased *O*-alkyl C indicated
579 dominance of microbial biomass products in mineral-associated SOC following litter removal.

580 Overall, our data indicate that most plant-derived compounds, of which phenolic and
581 aromatic C are indicative, are not retained in mineral associations over the long-term in humid
582 tropical forest soils. Rather, microbial biomass compounds like proteins and cell wall polymers
583 (containing *O*-alkyl and *N*-alkyl C groups), as well as lipids (alkyl C), appear to be preferentially
584 retained on mineral surfaces in these soils on a decadal timescale. Our ^{13}C NMR data also
585 suggest that a revised ratio of (alkyl C + *O*-alkyl C + *N*-alkyl):(aromatic + phenolic), may be a

586 useful indicator of the biochemical stability of SOC in strongly weathered tropical forest soils.
587
588 ***4.5. Different Seasonal Effects Across SOC Fractions***
589 Our data indicate that dry-season declines in mineral-associated and occluded-debris
590 SOC explain an observed overall decrease in bulk soil C stocks during the dry season at these
591 sites (Turner et al., 2015). The wet season increase in mineral-associated SOC, particularly with
592 litter addition, may indicate the importance of rainfall leaching DOC from litter downward and
593 subsequent DOC sorption to mineral surfaces (Qualls et al., 2002). The wet-season increase in
594 occluded-debris SOC could be related to elevated formation and dispersal of macro aggregates (2
595 – 4.75 mm diameter), which is common during wet periods in seasonally dry sites (Bach and
596 Hofmockel, 2016). In contrast, the wet-season decline in free-debris SOC likely reflects a two-
597 fold increase in forest floor decomposition rates during the wet season in these Panamanian
598 forests relative to the dry season (Wieder and Wright, 1995). Overall, our data suggest a seasonal
599 transfer of SOC between fractions, with added litter incorporated into free-debris space during
600 the dry season, and then possibly transferred to occluded-debris and/or mineral associated
601 fractions during the subsequent wet season, along with C from new litter.
602
603 **5. Conclusion**
604 This study illustrates that the physical and biochemical nature of soil C stocks will be
605 sensitive to changes in tropical forest NPP with global change. Most notably, the relatively stable
606 mineral-associated SOC fraction showed large changes with a decade of litter manipulation.
607 Litter addition promoted the accumulation of C into organo-mineral associations that were
608 relatively stable (i.e. not leachable as DOC), suggesting that strongly weathered tropical soils

have the capacity to store more C if tropical forest NPP increases. Litter removal demonstrated that the most stable portion of mineral-associated SOC had elevated dominance of lipids like waxes (alkyl C), and microbial biomass products like proteins and cell walls (*N*-alkyl and *O*-alkyl C). In contrast, plant-derived compounds, characterized by aromatic and phenolic C, formed a more dynamic portion of mineral-associated SOC, demonstrating that these may have limited importance for long-term soil C storage in strongly weathered tropical soils. Seasonal patterns indicated an accumulation of free-debris SOC during the dry season, followed by occluded-debris and mineral-associated SOC increases during the wet season, promoting an overall increased in bulk soil C during the wet season. Thus, a change in the duration or severity of the dry season may interact with changes in tropical forest NPP to alter soil C storage in tropical forests. For example, NPP-driven increases in mineral-associated SOC could diminish under drying scenarios. Overall, our findings show that changes in tropical forest NPP are likely to cause large changes in the quantity, stability, and biochemical character of soil C stocks.

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Figure Legends

Figure 1. (**A.**) Bulk soil C concentrations, made up of three SOC fractions, are shown for litter addition (L+), control (C), and litter removal (L-) plots during a wet and dry season (0 – 5 cm depths). Bulk soil C concentrations were significantly greater in L+ versus C and L-, driven primarily by greater mineral-associated SOC. (**B.**) Free-debris SOC was greater in L+ versus C and L-, with the largest treatment effect during the dry season. (**C.**) Occluded-debris SOC was greater in L+ versus L-, with the largest treatment effect during the wet season. ^ indicates significantly greater free-debris SOC during the dry season; # indicates significantly greater mineral-associated and occluded-debris SOC fractions during the wet season. Tukey HSD means separation results are shown in capital letters for bulk soil C concentrations, and lower case letters for SOC fractions, showing differences across both seasons. Averages are shown for each fraction ± one SE for that fraction (n = 5).

Figure 2. Average radiocarbon ($\Delta\ ^{14}C$) values for mineral-associated SOC are shown for litter addition (L+), control (C), and litter removal (L-) plots. All $\Delta\ ^{14}C$ values reflect ^{14}C released during bomb testing in the 1960s (i.e., "modern" values), so lesser $\Delta\ ^{14}C$ values in L+ versus L- indicate more retention of recent C inputs into mineral-associated SOC with litter addition. Letters shown in the bars indicate Tukey HSD differences in the mean among the treatments. There were no seasonal effects so values were pooled. Averages are shown ± one SE (n = 5).

Figure 3. Representative ^{13}C NMR spectra are shown for mineral-associated SOC in a litter removal plot (black line, L-), and a litter addition plot (gray line, L+). Chemical shift regions shown on a unitless δ-scale represent seven SOC functional groups: alkyl (0-45 ppm), *N*-

alkyl+methoxyl (45-60 ppm), *O*-alkyl (60-95 ppm), di-*O*-alkyl (95-110 ppm), aromatic (110-145 ppm), phenolic (145-165 ppm), and amide+carboxyl (165 – 215 ppm). The proportion of total SOC in the alkyl, N-alkyl and O-alkyl regions was significantly after a decade of litter removal versus addition (Table 2).

Figure 4. The proportion of C leached as DOC is shown for (**A.**) free-debris SOC, and (**B.**) mineral-associated SOC, comparing litter addition (L+), control (C), and litter removal (L-) plots. (**A.**) A greater proportion of free-debris SOC was leachable as DOC in L- versus L+. (**B.**) In contrast, a greater proportion of the mineral-associated SOC was leachable as DOC in L+ versus L-, suggesting greater C desorption. Letters indicate Tukey HSD differences in the mean among the treatments. There were no seasonal effects so values were pooled. Averages are shown ± one SE (n = 5).

888 **Tables**

889 **Table 1:** Effects of litter manipulation on bulk soil chemistry and soil characteristics are shown for a wet and a dry season in Panama

890 (0 – 5 cm depth). Averages are given ± one SE (n = 5).

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Season	Treatment	Bulk Soil %C [a]	Bulk Soil %N [a]	Bulk Soil C:N ratio [a]	Soil Moisture g-water/ g-soil [a, b, c]	Bulk Density (g/cm^3) to 5 cm depth [a]	C Stock (mg/cm^2) to 5cm depth [a]	Leachable DOC mg-C/g-soil [a]	Leachable TDN µg-N/g-soil [a]
Dry	Addition	6.7 ± 0.5	0.63 ± 0.02	10.7 ± 0.8	0.43 ± 0.03	0.82 ± 0.08	269 ± 20	1.1 ± 0.3	254 ± 38
	Control	5.2 ± 0.3	0.52 ± 0.02	9.9 ± 0.3	0.42 ± 0.01	0.78 ± 0.03	200 ± 14	0.9 ± 0.1	213 ± 40
	Removal	3.6 ± 0.2	0.38 ± 0.01	9.4 ± 0.4	0.35 ± 0.01	0.99 ± 0.04	180 ± 15	0.5 ± 0.04	128 ± 5
Wet	Addition	7.3 ± 0.6	0.61 ± 0.04	12.0 ± 0.8	0.72 ± 0.02	0.76 ± 0.02	278 ± 25	1.6 ± 0.3	227 ± 35
	Control	5.4 ± 0.3	0.51 ± 0.02	10.5 ± 0.3	0.66 ± 0.02	0.68 ± 0.03	184 ± 15	0.9 ± 0.1	150 ± 15
	Removal	3.7 ± 0.2	0.38 ± 0.02	9.6 ± 0.4	0.52 ± 0.01	0.95 ± 0.06	175 ± 16	0.6 ± 0.1	111 ± 17

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a significant effect of treatment

b significant effect of season

c interacting effect of treatment and season

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Table 2. The percentages of SOC belonging to seven C functional groups as detected using ^{13}C NMR are shown for the mineral-associated SOC fraction in litter addition, control, and litter removal plots. Examples of common environmental organic compounds containing each C group are listed in parentheses, and chemical shift regions of the spectra are given for each C group. There were no seasonal effects so data are pooled for two seasons. Averages are given ± one SE (n = 5).

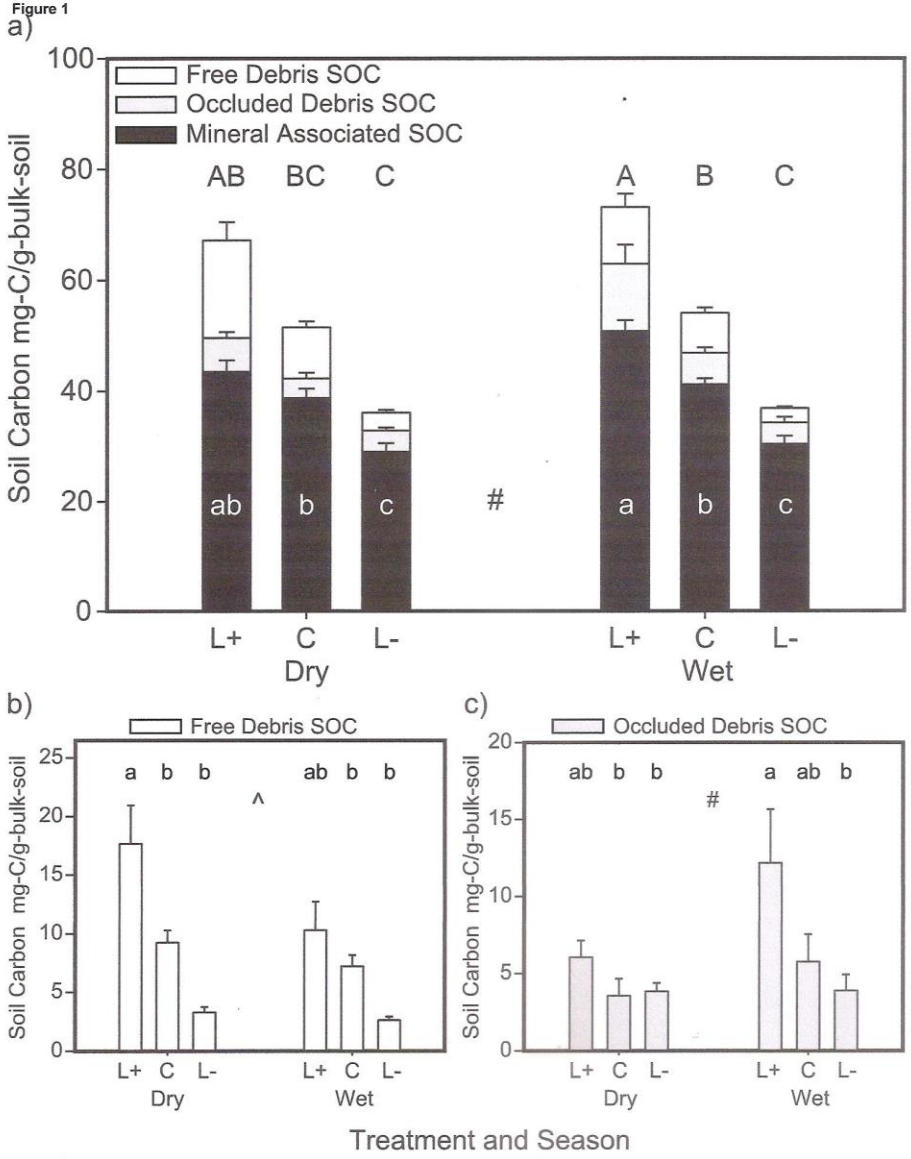
Plant Litter Treatment	**Alkyl C (waxes, other lipids) 0-45 ppm** [a]	***N*-Alkyl + Methoxyl C (proteins, peptides) 45-60 ppm** [a]	***O*-Alkyl C (cellulose, other carbohydrates) 60-95 ppm** [a]	**Di-*O*-Alkyl C (hemicellulose) 95-110 ppm** [a, b]	**Aromatic C (lignin, tannin) 110-145 ppm** [a]	**Phenolic C (acids, tannin) 145-165 ppm** [a]	**Amide + Carboxyl C (chitin + hemicellulose) 165-215 ppm** [b]	**Ratio of (Alkyl+*O*-alkyl +*N*-alkyl) :(phenolic + aromatic) C** [a]
Addition	25.3 ± 0.5	9.2 ± 0.2	18.2 ± 0.4	7.3 ± 0.3	17.4 ± 0.4	7.9 ± 0.2	14.7 ± 0.5	2.1 ± 0.08
Control	25.4 ± 0.3	9.7 ± 0.2	19.5 ± 0.3	7.0 ± 0.2	16.4 ± 0.3	7.2 ± 0.1	14.8 ± 0.4	2.3 ± 0.06
Removal	28.4 ± 0.5	10.3 ± 0.2	21.1 ± 0.5	6.4 ± 0.2	14.3 ± 0.4	5.9 ± 0.3	13.6 ± 0.4	3.0 ± 0.1

a Significant effect of litter manipulation.

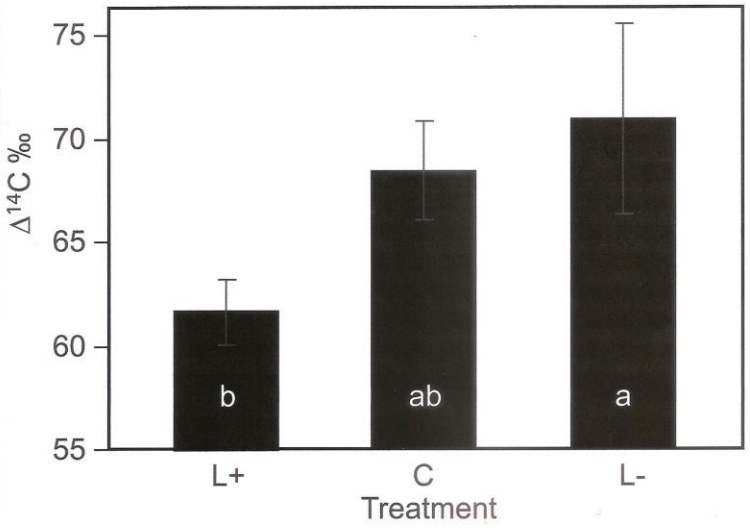
b While cellulose and hemicellulose are both examples of carbohydrates, which are built from *O*-Alkyl C, hemicellulose contains carboxyl C and can thus be distinguished in NMR spectra. Glucoronic acid is a major constituent of hemicelluloses in gymnosperms

and angiosperms, and glucoronic acid contains carboxyl C. The *N*-Acetyl glucosamine polymer (chitin) is the primary cell wall component of fungi, and as the name indicates, chitin contains acetyl groups. Acetyl C is a type of carboxyl C. Therefore, both hemicellulose and chitin contain carboxyl C in measurable quantities. Hemicellulose and chitin are both carbohydrates and both cell wall components of abundant organisms in the ecosystem.

Figure 1


a)
Free Debris SOC
Occluded Debris SOC
Mineral Associated SOC
Soil Carbon mg-C/g-bulk-soil
AB BC C A B C
ab b c # a b c
L+ C L- L+ C L-
Dry Wet
b)
Free Debris SOC
a b b ab b b
^
c)
Occluded Debris SOC
ab b b a ab b
#
Treatment and Season

igure 2



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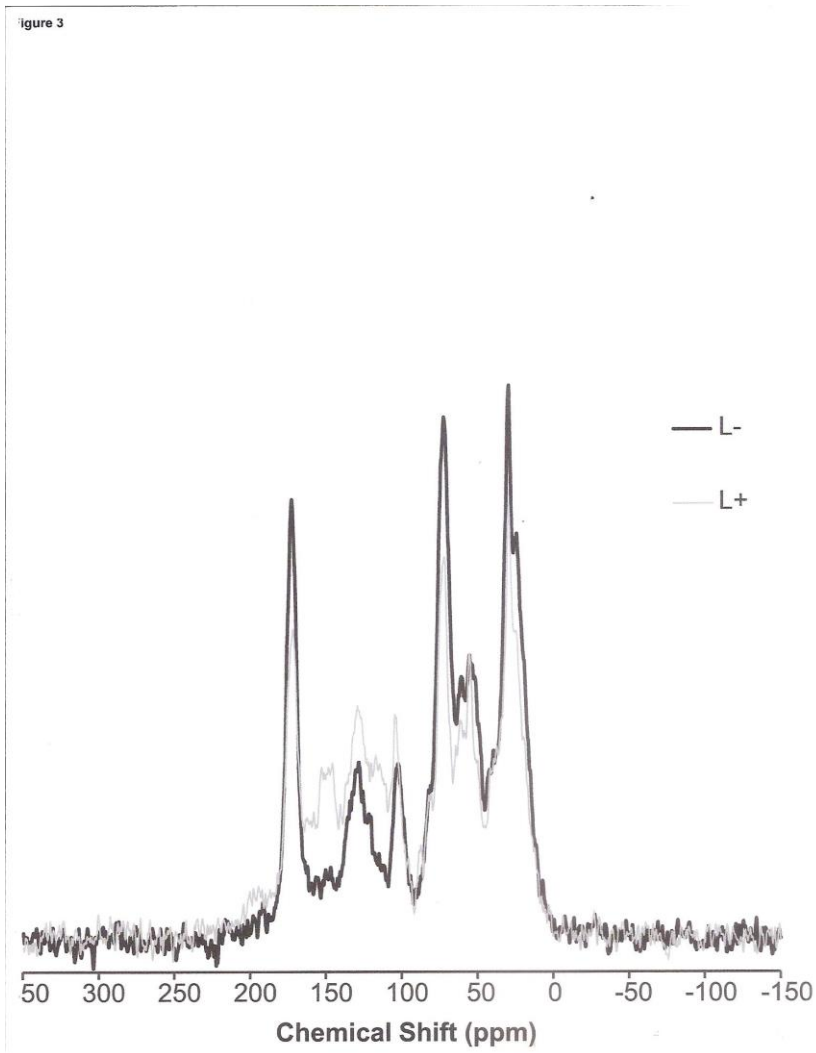
igure 3
L-
L+
50 300 250 200 150 100 50 0 -50 -100 -150
Chemical Shift (ppm)

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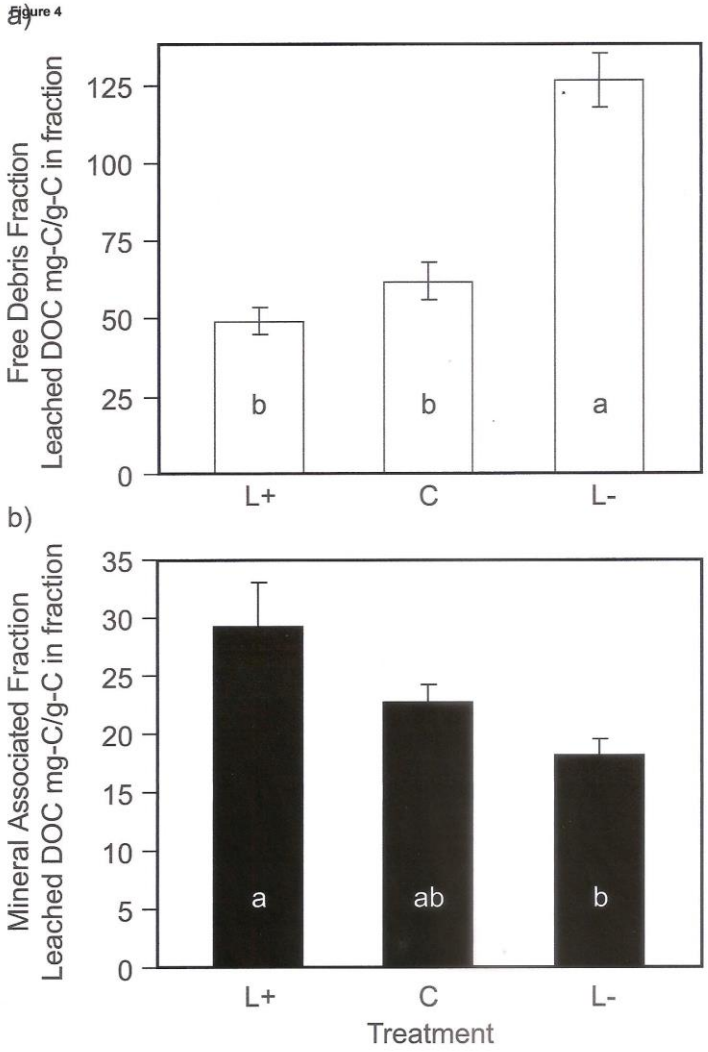
a)
Free Debris Fraction
Leached DOC mg-C/g-C in fraction
125
100
75
50
25
0
b
b
a
L+
C
L-
b)
Mineral Associated Fraction
Leached DOC mg-C/g-C in fraction
35
30
25
20
15
10
5
0
a
ab
b
L+
C
L-
Treatment

Figure 4

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Figure S2

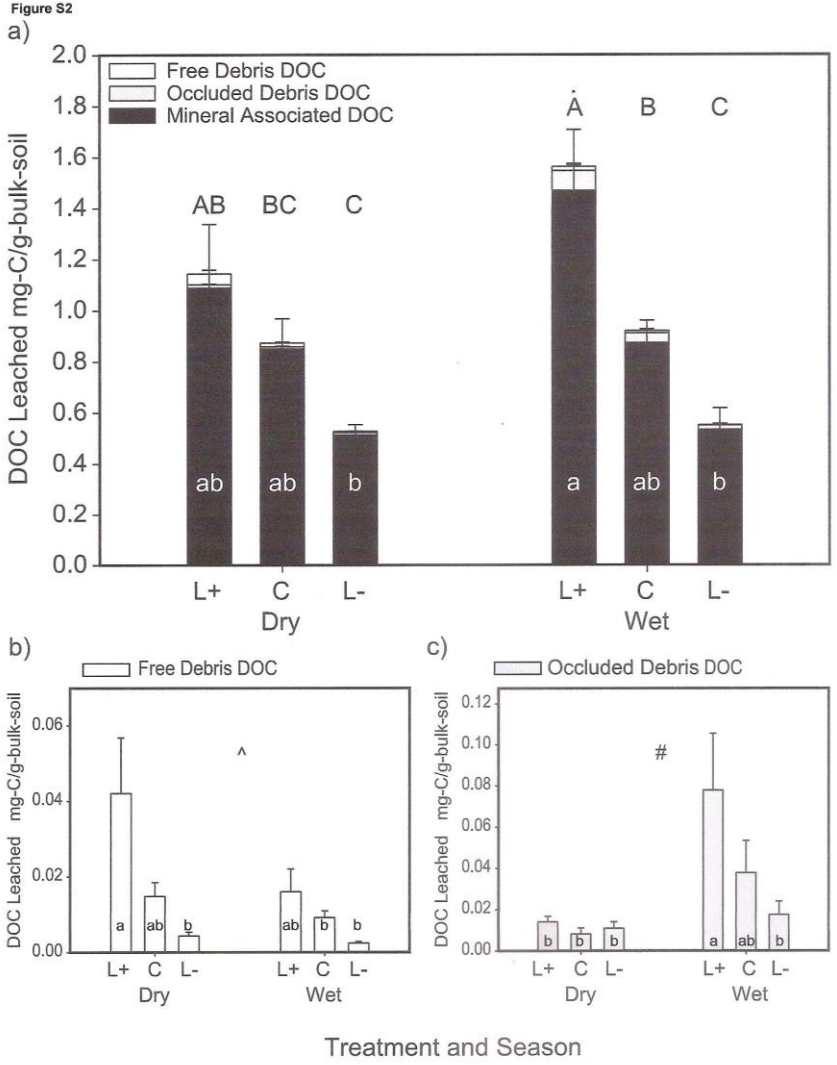

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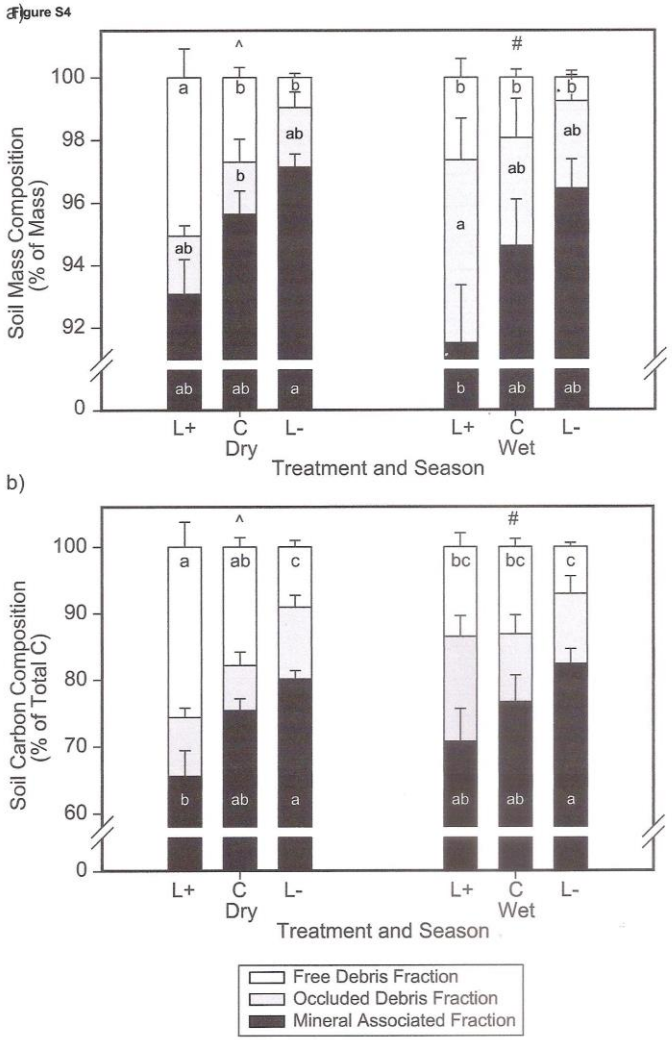
Figure S4



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